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## **Lignin content and chemical characteristics in maize and wheat vary between plant organs and growth stages: consequences for assessing lignin dynamics in soil**

Abiven, S ; Heim, A ; Schmidt, M W I

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# Lignin content and chemical characteristics in maize and wheat vary between plant organs and growth stages: consequences for assessing lignin dynamics in soil

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**Abstract** Assessing lignin turnover in soil on the basis of a  $^{13}\text{C}$  natural abundance labeling approach relies on the assumption that chemical characteristics of labeled and control plant inputs are similar and that the  $^{13}\text{C}$  content difference between labeled and control plant inputs is constant within the plant parts. We analyzed lignin in soils, roots, stems and leaves of wheat and maize at different stages of growth using the cupric oxide oxidation method. In both plants, lignin concentrations increased with growth, particularly during grain filling. Maize contained more cinnamyl moieties than wheat. Roots had higher lignin contents (especially cinnamyl moieties) than stems and leaves, and seemed to contribute more to the total soil lignin than the aboveground parts. The isotopic differences ( $\Delta \delta^{13}\text{C}$ ) of lignin phenols were not significantly different ( $p > 0.05$ ) between plant organs, confirming assumptions underlying the natural abundance  $^{13}\text{C}$  labeling approach. Our data show that lignin content and phenol distribution can vary between plant organs and with the time of harvest. Consequently, the amount of annual lignin input may vary as a function of root amount and

harvest date, and thus can affect the calculated apparent turnover times of lignin in natural abundance  $^{13}\text{C}$  labeling experiments.

**Keywords** Soil lignin turnover · C3-C4 natural abundance labeling · Plant composition · Roots

## Introduction

The term lignin covers a group of plant aromatic polymers of methoxylated phenylpropanoids connected by both ether and carbon-carbon linkages (Ralph et al. 2004). Lignin contributes significantly to the organic carbon input to soil (Kögel-Knabner 2002). Due to the abundance of aromatic structures suggesting chemical recalcitrance, lignin was considered until recently as a major contributor to the soil organic matter (Thevenot et al. 2010). However, based on a C tracing method that exploits the difference in the ratio of  $^{13}\text{C}$ : $^{12}\text{C}$  in  $\text{C}_4$  plants (e.g. maize) and  $\text{C}_3$  plants (e.g. wheat) (Balesdent and Mariotti 1987; Thevenot et al. 2010), lignin monomers were found to turn over faster than bulk soil organic carbon (Dignac et al. 2005; Heim and Schmidt 2007a). However, the natural abundance isotope labeling approach relies on several assumptions:  $\text{C}_3$  and  $\text{C}_4$  plants are identical regarding (1) the quantity, (2) the chemical characteristics, and (3) the distribution of the lignin within the plant, and (4) the distribution of the  $^{13}\text{C}$  isotope within the lignin in the different plant parts.

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Lignin quantity has been shown to vary with plant maturity, and with plant species (Crampton and Maynard 1938; Knudsen 1997; Palm et al. 2001), as well as between individuals of the same species (Whetten and Sederoff 1995). Comparing plant material used for forage (e.g. *Medicago sativa*, *Cynodon dactylon*, *Panicum maximum*, *Avena sativa* (Fukushima and Dehority 2000; Hatfield and Fukushima 2005) and *Andropogon gerardii* (Titgemeyer et al. 1995)) at young and mature stages, several authors observed a significant increase in lignin content with plant maturity.

Plant lignin chemical characteristics differ between species, e.g. between angiosperms and gymnosperms (Higuchi 1985; Whetten and Sederoff 1995), or between maize and wheat (Dignac et al. 2005; Heim and Schmidt 2007a). The evolution of the lignin composition with growth has not yet been intensively studied. Morrison et al. (1998) and Titgemeyer et al. (1995) reported an increase in cinnamyl phenol moiety concentration with maturation. Differences in lignin content were also reported between plant parts, with a higher lignin content in roots than in stems and leaves (Dignac et al. 2005; Heim and Schmidt 2007a), reflected in the decomposition rate of plant residues (Abiven et al. 2005). Lignin in wheat roots appeared richer in cinnamyl moieties and consisted of more condensed structures than lignin in above ground parts (Bertrand et al. 2006).

The natural abundance of the  $^{13}\text{C}$  isotope has been reported as differing between the individual subunits of lignin and between plant parts: for example, cinnamyl phenol moieties were reported as depleted in  $^{13}\text{C}$  compared to vanillic and syringic phenol moieties in spruce species (Göni and Eglinton 1996). Also, differences were observed between different wood species like spruce, beech or poplar (Göni and Eglinton 1996). Within maize and wheat plants, Dignac et al. (2005) observed more negative  $\delta^{13}\text{C}$  values for lignin in leaves than in stems and roots.

Thus, different chemical and isotopic compositions and concentrations of individual lignin monomers exist, and therefore may affect turnover calculations. The purpose of this paper was (i) to quantify lignin and characterize its chemical and isotopic properties in roots, stems and leaves of maize and wheat at several growth stages, (ii) to compare its quantity and quality with lignin in mineral soil, and (iii) to evaluate the importance of the variation in lignin inputs for the

turnover calculations based on the assumption that lignin inputs are not different.

## Material and methods

### Soil and plant samples

Plants were sampled in 2005 at the Rothalmünster experimental site (full description by Ludwig et al. 2005), located in a rural area in Southern Germany. The mean annual temperature in the region is 8.2°C and the mean annual precipitation 890 mm. At the site, grassland was established until 1960 and then different  $\text{C}_3$  plants (summer wheat followed by winter wheat—*Triticum aestivum* v. *Petrus*) were grown. In 1979, the plot was reorganized as follows: (i) wheat was continued on one part of the plot and (ii) a maize (*Zea mays* L. v. *Anjou 219*) monoculture was established on the other part. The soil type is a Stagnic Luvisol developed from loess (10% sand, 73% silt, 17% clay). Soil samples from 0 to 30 cm were taken in September 2002. Prior to the analysis, the soil was dried at 40°C for 24 h and sieved at 2 mm. Further details about the soil can be found in Heim and Schmidt (2007b).

Plants were sampled from both plots during the 2005 vegetative season. Whole plants of wheat and maize were collected at 4 and 5 dates, respectively, corresponding to different degrees of maturity (Table 1). The sampling period corresponds to the vegetative phase and to grain filling (between the two last sampling dates) of the plants. At each date, 4 to 5 plants were sampled and plant organs of these 4–5 plants were pooled together. The samples were air dried during 72 h immediately after the sampling in order to avoid decay of the material and separated into roots, stems and leaves. Leaves and stems could not be distinguished for maize samples collected on the 30<sup>th</sup> of May, so they were pooled. The roots were sampled with a shovel within the first 30 cm of the soil, carefully separated from the soil and washed with water. Both fine and large roots were sampled and pooled prior to analysis. However, the proportion of fine roots might be underestimated due to the sampling method.

Three replicates (from the pooled plant organ samples) were analyzed for each date and each organ. Plant samples were oven dried at 40°C for 24 h and milled mechanically prior to analysis.

**Table 1** Dates of sampling and corresponding date after sowing (DAS), heat sum and phenological stage for wheat (a) and maize (b). Heat sum is the sum of the daily average temperature, describing the phenological stages of the maize and wheat

Dates	DAS	Heat sum	Phenological stage
a. Wheat			
14-Oct. 2004	0	0	Sowing
25-Apr. 2005	193	402	Second node
30-May 2005	228	850	Flag leaf fully emerged
30-Jun. 2005	259	1,364	Milky ripe
30-Jul. 2005	289	1,796	Maturity
b. Maize			
2-May	0	0	Sowing
30-May	28	200	–/+ 5 leaves
27-Jun	56	498	8 to 9 leaves
27-Jul	86	874	Blooming + 5 days
1-Sep	122	1,248	Seed 65% water content
15-Oct	166	1,550	Seed 40% water content

### Chemical analysis

Bulk organic carbon concentration in soils and plants was determined using a CHN analyzer (Vario EL, Elementar Analysis systems, Hanau, Germany). Dried and milled soil and plant samples were subjected to alkaline CuO oxidation (Hedges and Ertel 1982) in order to break lignin molecules into monomers. We used microwave digestion as described by Goñi and Montgomery (2000), with modifications as described below. All samples were oxidized in triplicate using a microwave oven (ETHOS EM-2: 1,000 W nominal power; Egrolyt AG, Oberwil, Switzerland). Soil and plant samples equivalent to 2–5 mg C were weighed and oxidized with 500 mg CuO powder, 50 mg of ferrous ammonium sulfate and 20 ml 2 M NaOH solution in the microwave at 150°C for 90 min. After the vessels had cooled, an internal standard of 500 µl of cinnamic acid + ethylvanillin mixture (50 mg L<sup>-1</sup> each) was added to each sample. Humic acids were removed by precipitation after acidification of the solution to a pH of ca. 2 with concentrated HCl. The samples were collected on preconditioned (Ethyl Acetate (EtAc), Methanol, H<sub>2</sub>O) C<sub>18</sub> columns, from which they were eluted with 5×500 µl EtAc and dried under a stream of N<sub>2</sub>. The dried samples were dissolved in 400 µl anisic acid in EtAc (50 mg l<sup>-1</sup>).

For each solution, the individual lignin monomers were quantified in triplicate after derivatization of 70 µl sample with 70 µl N,O-bis (trimethylsilyl) trifluoroacetamide/tetramethylchlorosilane (BSTFA/TMCS; 99:1; Fluka, Buchs, Switzerland) for 15 min at 60°C. Analysis was performed using gas chromatography (GC; Agilent 6890) with flame ionization detection (FID) as follows: column DB-5, 50 m×200 µm, 5 m pre-column, i.d. 0.32 mm, film thickness 0.33 µm (Agilent J & W, Folsom, CA, USA); temperature program, 100–160°C at 3°C min<sup>-1</sup>, to 320°C at 10°C min<sup>-1</sup>, hold 10 min. Peak heights were quantified using external calibration curves. The data were corrected for losses during sample preparation. For each sample, the average recovery of the internal standards ethylvanillin and cinnamic acid (typically 60–80%) was determined individually, and the raw data divided by the recovery.

Eight lignin oxidation products were quantified. The concentration of V-type lignin was calculated as the sum of the concentrations of vanillin, acetovanillone and vanillic acid. The concentration of S-type lignin was calculated as the sum of the concentrations of syringaldehyde, acetosyringone and syringic acid. C-type lignin was calculated as the sum of ferulic and *p*-coumaric acids. The ratios S/V, C/V, C/S were calculated to estimate changes in lignin chemical characteristics.

Compound specific isotope analysis (CSIA) was performed at least in duplicate for each plant sample extract from the last sampling date (harvest). Samples were separated with a HP 6890 gas chromatograph [Palo Alto, USA, column: Varian Factor four VF-5 ms (Varian, Darmstadt, Germany), length 60 m, i.d. 0.25 mm, film thickness 0.33 mm; temperature program: 100 °C (2 min) to 250°C at 3°C min<sup>-1</sup> to 300°C (hold 10 min) at 20°C min<sup>-1</sup>], combusted at 940°C and the CO<sub>2</sub> was analyzed using a Delta plus XP isotope ratio mass spectrometer (IRMS—Thermo Finnigan MAT, Bremen, Germany). Combustion water was trapped with a 0.3 mm i.d. Nafion<sup>TM</sup> capillary. We added C<sub>20</sub> and C<sub>24</sub> *n*-alkanes to the samples as internal standards for CSIA (Heim and Schmidt 2007a). The δ<sup>13</sup>C values of these alkanes, determined using an elemental analyzer (EA) coupled to an isotope ratio mass spectrometer, were used to correct for any isotopic shift during analysis. Alkanes were used for this purpose because they do not require derivatization, so the isotopic value deter-

mined using gas chromatography-combustion-isotope ratio mass spectrometry (GC-C-IRMS) can be directly compared to those obtained using EA-IRMS.

The  $\delta^{13}\text{C}$  monomers isotopic values were corrected from the error introduced by the BSTFA derivatization, as proposed by Dignac et al. (2005). Briefly, the BSTFA  $\delta^{13}\text{C}$  value measured off line was used to correct the  $^{13}\text{C}$  concentrations of the monomers measured in the samples with GC/C-IRMS, according to the following Eq. 1:

$$\delta_{\text{UD}} = \frac{n_{\text{D}}\delta_{\text{D}}}{n_{\text{UD}}} - \frac{n_{\text{BSTFA}}\delta_{\text{BSTFA}}}{n_{\text{UD}}} \quad (1)$$

where  $\delta_{\text{UD}}$  is the isotopic ratio of the underivatized phenol,  $n_{\text{UD}}$  is the number of carbon atoms in the underivatized phenol,  $\delta_{\text{BSTFA}}$  is the isotopic ratio of BSTFA,  $n_{\text{BSTFA}}$  is the number of carbon atoms added from BSTFA,  $\delta_{\text{D}}$  is the isotopic ratio of the derivatized phenol and  $n_{\text{D}}$  is the number of carbon atoms in derivatized phenol.

#### Data analysis

Standard errors of derived variables were calculated by way of error propagation from the standard errors determined from the replicate extractions (see above).

Analysis of variance (ANOVA) was used following a General Linear Model (GLM) of R software (R Development Core Team 2008) to determine differences attributable to treatments. A least significant difference analysis (LSD,  $p \leq 0.05$ ) was then performed as post-hoc test to the ANOVA for the concentrations of lignin and the ratios between the lignin monomers at each sampling date for all treatments (plant part). Student *t*-test was used to compare the values of the same treatment at two dates ( $p \leq 0.05$ ).

#### Turnover modeling

To estimate the consequences of our observations on the lignin monomers turnover calculation in soil, we used a simple 2 pool-model already described by Heim and Schmidt (2007b). Briefly, this model describes a fast and a slow decomposing pools of lignin in soil over 25 years. Apparent turnover times were estimated from maize—derived lignin with the

following formula (2), which assumes mono-exponential decay:

$$T = -t/\ln(1 - F), \quad (2)$$

where  $T$  = apparent turnover time of the lignin,  $t$  = duration of maize cultivation and  $F$  the the proportion of the maize-derived lignin in the soil.

The model has been calibrated for the same site studied here, so we used it with the same parameterization. The fast and slow pools had a decomposition rate equal to  $1.88 \text{ yr}^{-1}$  and  $0.052 \text{ yr}^{-1}$ , respectively.

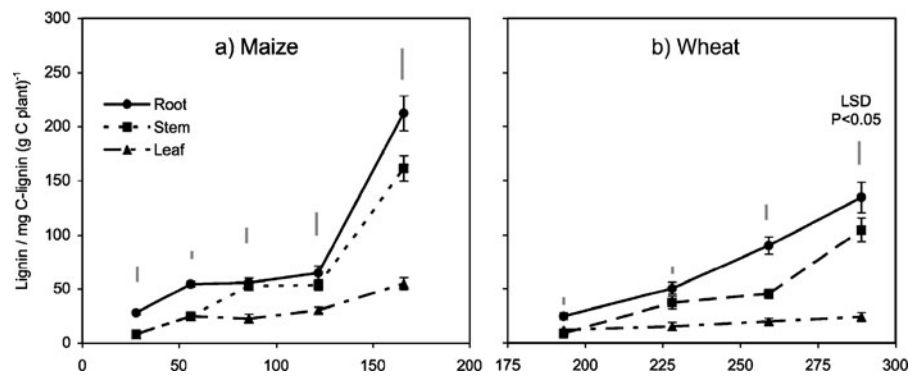
We tested the potential effect of harvest date (and thus the resulting quantity of lignin input into the soil) on the lignin turnover calculation using three scenarios of lignin input. First, as a baseline we used the real harvest date. Second, we used an input corresponding to harvest 2 weeks before the real harvest date. Third we used an input of lignin corresponding to harvest 4 weeks before the real harvest date. The data for these two last scenarios were calculated by a linear extrapolation of the data measured for the one but last and the last sampling dates. Such a variation of up to 1 month seems realistic, since at this physiological stage of the grain filling the actual harvest dates typically vary with weather and farm management. Dry matter of stems, leaves and roots typically stays constant or tends to decrease during the last month before harvest. The lignin input was calculated from our measured data (Fig. 1a) and from a previous yield estimation (Ludwig et al. 2005):  $99.0$ ,  $66.7$  and  $34.3 \text{ g lignin C m}^{-2} \text{ y}^{-1}$  for the real harvest date, 2 weeks before and 4 weeks before respectively. Since we did not find any variation in the isotopic composition of the lignin monomers, we did not introduce differences in the isotopic content of the lignin monomers in the modelisation.

## Results

#### Changes in lignin concentrations and quality during plants growth

Total lignin concentrations in each part of both plant species increased with maturation (Fig. 1), from  $11.7 \pm 1.1 \text{ mg C lignin gC plant}^{-1}$  at day 193 to  $23.8 \pm 4.2 \text{ mg C lignin gC plant}^{-1}$  at day 289 for the wheat leaves (smallest increase,  $p < 0.001$ ) and from  $8.0 \pm 0.9 \text{ mg C lignin gC plant}^{-1}$  at day 28 to  $161.1 \pm$

**Fig. 1** Lignin concentrations (mg C-lignin (g C-plant)<sup>-1</sup>) in roots (*circles*), stems (*squares*) and leaves (*triangles*) for maize (**a**) and wheat (**b**) along growth of the plant (days after sowing). The gray bars represent the LSD ( $p<0.05$ ) for each sampling date. Average values calculated from 3 replicates, black bars represent the standard errors

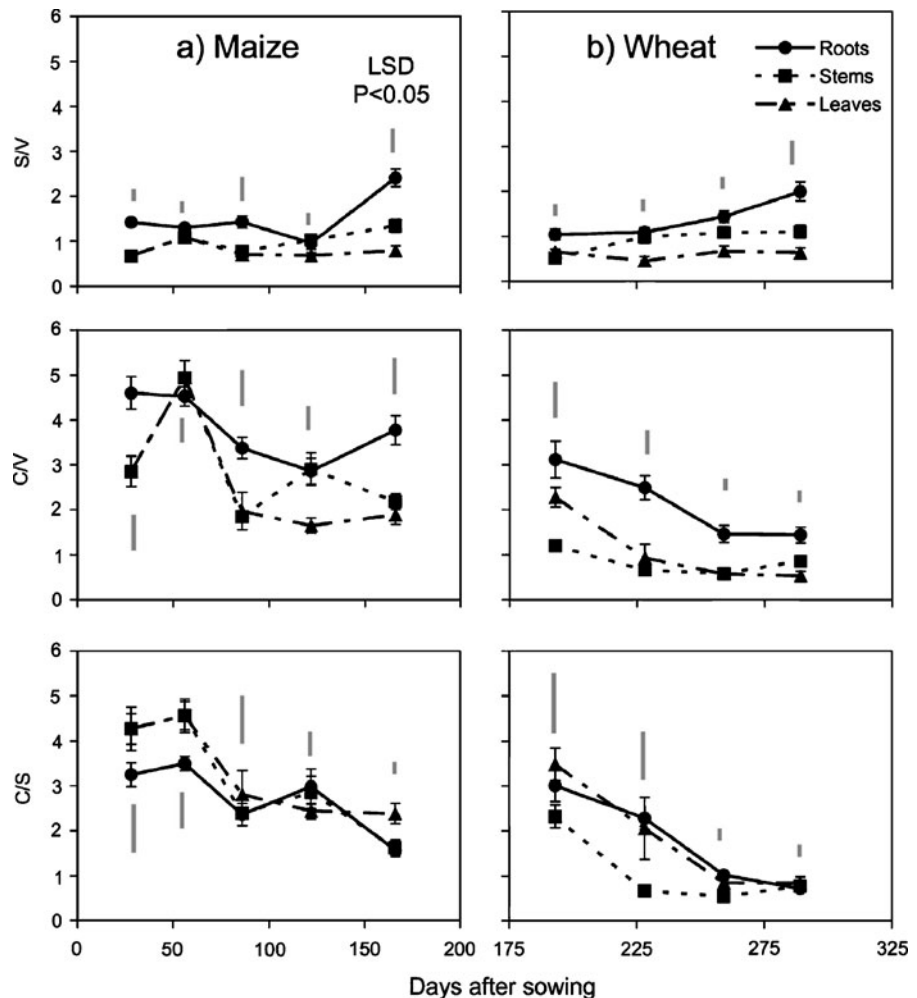


11.6 mg C lignin gC plant<sup>-1</sup> at day 166 for the wheat leaves (largest increase,  $p<0.001$ ). The increase, though, was more important in maize roots and stems, and in wheat stems at the final stage of maturation (i.e. by a factor of 3.3 between day 122 ( $64.7\pm6.5$  mg C lignin gC plant<sup>-1</sup>) and day 166 ( $212\pm16.1$  mg C lignin

g C plant<sup>-1</sup>) in maize roots,  $p<0.001$ ) than for the other plant organs, which has not been reported previously. The last stage corresponds to grain filling (Table 1) and allocation of reserve material to the fruit.

Figure 2 illustrates composition changes in lignin during plant growth. During growth of both plants, C/

**Fig. 2** Ratios of syringyl (S), cinnamyl (C) and vanillin (V) phenol type in roots (*circle*), stems (*square*) and leaves (*triangle*) maize and wheat during the plant growth (days after sowing). The gray bars represent the LSD ( $p<0.05$ ) for each sampling date. Average values calculated from 3 replicates, black bars represent the standard errors





V and C/S ratios decrease (largest decrease in wheat leaves, from  $2.3 \pm 0.2$  to  $0.5 \pm 0.1$  for C/V ( $p < 0.001$ ) and from  $3.5 \pm 0.2$  to  $0.8 \pm 0.1$  for C/S ( $p < 0.001$ )), whereas S/V ratio remains constant or increases (largest increase in wheat stems, from  $0.5 \pm 0.1$  to  $1.1 \pm 0.1$  ( $p < 0.001$ ), reflecting the preferential deposition of V and S units at later stages of maturity. This is in accord with observations that syringyl moieties are formed later during maturation (Morrison et al. 1998; Ralph et al. 2004).

#### Lignin concentrations in different plant parts

In both plants, roots consistently contain more lignin than stems and leaves during the entire growth period (Fig. 1 and Table 2). At the last date of sampling, maize stems ( $161.1 \pm 11.1$  mg C lignin g C plant<sup>-1</sup>) and roots ( $212.0 \pm 16.1$  mg C lignin g C plant<sup>-1</sup>) contained 3 and 4 times more lignin than maize leaves ( $55.0 \pm 5.5$  mg C lignin g C plant<sup>-1</sup>,  $p < 0.001$ ), respectively. In wheat stems ( $104.6 \pm 11.2$  mg C lignin g C plant<sup>-1</sup>) and roots ( $134.5 \pm 13.8$  mg C lignin g C plant<sup>-1</sup>), lignin content was 4.4 and 5.6 times higher ( $p < 0.001$ ) than in wheat leaves ( $23.8 \pm 4.2$  mg C lignin g C plant<sup>-1</sup>).

#### Differences between species in lignin content

In the present study, at harvest, maize contained more lignin than wheat (Fig. 1 and Table 2). This was also observed by Heim and Schmidt (2007a), whereas Bahri et al. (2006) and Dignac et al. (2005) observed the opposite. This difference could be due to different ripening stages, which appear critical according to our data (Fig. 1). We observed that differences between the two plants occur only during the final stage of maturation. Maize lignin contains more cinnamyl moieties than wheat (>50% of lignin monomers, Table 2), as reflected in the C/V and C/S ratios (Fig. 2). S/V ratio values are comparable for both plants.

#### Comparison between plant and soil lignin

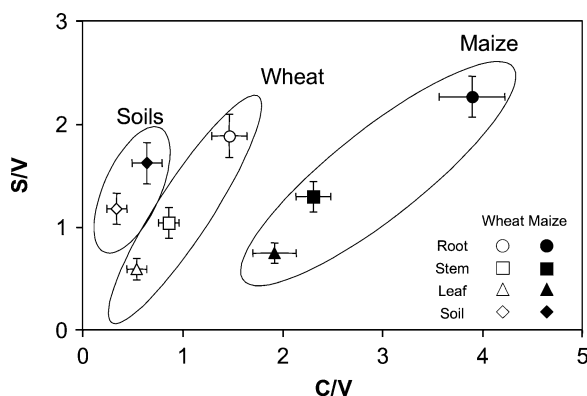
Soil lignin monomer concentrations were lower for the wheat plot ( $18$  mg C lignin (g OC soil)<sup>-1</sup>) than for the maize plot ( $24$  mg C lignin (g OC soil)<sup>-1</sup>).

In Fig. 3, C/V is plotted against S/V, for comparing data for soils and harvested biomass. We observed lower C/V values for the soils than for plants due to a preferential loss of C units, which is

**Table 2** Lignin monomers content and corrected isotopic values of wheat and maize roots, stems and leaves at the last sampling date (harvest date)

	Vl	Vn	Vd	Sl	Sn	Sd	pCd	Fd
Lignin monomer content (mg C lignin. kg <sup>-1</sup> C-plant)								
Corrected lignin monomers isotopic value (δ <sup>13</sup> C, ‰)								
Maize root	28.0 (1.6)	0.6 (0.1)	1.0 (0.1)	58.2 (4.0)	6.5 (0.7)	2.3 (0.2)	93.0 (7.6)	22.3 (1.9)
	-15.7 (0.1)	-15.6 (1.3)	-17.8 (1.0)	-16.2 (0.1)	-17.0 (1.8)	-22.8 (2.0)	-19.1 (0.7)	-17.8 (1.1)
Maize stem	23.7 (2.4)	1.3 (0.0)	10.0 (1.0)	35.8 (3.5)	6.0 (0.9)	3.5 (0.5)	64.9 (2.5)	15.9 (0.8)
	-16.7 (0.6)	-17.7 (1.3)	-16.0 (1.2)	-16.9 (0.9)	-19.8 (2.4)	-23.7 (2.8)	-19.7 (0.4)	-18.9 (1.1)
Maize leaf	12.6 (1.5)	1.3 (0.1)	1.0 (0.1)	3.5 (0.3)	5.2 (0.7)	2.5 (0.4)	15.1 (1.4)	13.7 (1.0)
	-18.5 (0.3)	-18.9 (0.9)	-23.3 (0.5)	-18.9 (0.8)	-20.9 (1.1)	-25.3 (1.3)	-19.4 (0.8)	-20.8 (0.4)
Wheat root	28.4 (2.5)	0.8 (0.1)	1.8 (0.3)	50.4 (3.8)	5.1 (0.9)	2.7 (0.4)	25.8 (2.3)	19.6 (3.5)
	-30.1 (0.1)	-31.6 (1.1)	-30.5 (1.3)	-31.7 (2.4)	-30.5 (0.9)	-34.1 (0.1)	-28.4 (2.0)	-29.5 (1.2)
Wheat stem	32.2 (3.9)	0.8 (0.2)	3.0 (0.3)	25.3 (2.9)	7.5 (0.8)	4.6 (0.8)	12.3 (1.0)	18.8 (1.3)
	-31.1 (0.4)	-32.3 (0.6)	-35.2 (0.9)	-32.6 (0.4)	-33.3 (1.2)	-37.3 (0.6)	-31.4 (0.4)	-29.8 (0.2)
Wheat leaf	8.6 (1.4)	1.2 (0.2)	1.3 (0.5)	2.6 (0.2)	2.0 (0.3)	2.0 (0.5)	3.0 (0.4)	3.0 (0.7)
	-32.4 (0.3)	-33.8 (0.9)	-37.2 (0.5)	-35.6 (0.8)	-35.1 (1.1)	-38.8 (1.3)	-32.1 (0.8)	-30.8 (0.4)

Vl vanillin; Vn acetovanillone; Vd vanillic acid; Sl syringaldehyde; Sn acetosyringone; Sd syringic acid; Cd p-coumaric acid; Fd ferulic acid. Values presented are mean values ( $n=3$ ), standard error between brackets



**Fig. 3** Comparison of plant (roots with circles, stems with squares and leaves with triangles) and soil (diamond) lignin S/V and C/V ratios. Maize soil and plants with black symbol and wheat soil and plants with white symbol. Average values calculated from 3 replicates, black bars represent the standard errors

consistent with previous observations (Bahri et al. 2006). The S/V ratio increased in the order leaves < stems < soils < roots. For soil, our S/V value is consistent with literature values for arable soil [1.7 in Kiem and Kögel-Knabner (2003), 1.5 in Bahri et al. (2006)].

Comparing lignin monomers  $\delta^{13}\text{C}$  values between plant parts

In Fig. 4 we report  $\delta^{13}\text{C}$  values for each monomer as relative differences ( $\Delta$ ) between values obtained for maize and corresponding wheat samples after mathe-

matical correction. The corrected values for each monomer and each plant part can be found in Table 2.

The  $\Delta \delta^{13}\text{C}$  values are not significantly different between plant organs (LSD,  $p > 0.05$ , Fig. 4), which means that the natural abundance  $\delta^{13}\text{C}$  labeling is not specific to individual plant organs. The  $\Delta \delta^{13}\text{C}$  between wheat and maize is larger for vanillin and syringyl phenols than for cinnamyl phenols, which we cannot explain yet. As a consequence, we considered that the relative proportion of plant organs to total inputs will not affect the isotopic label of the inputs.

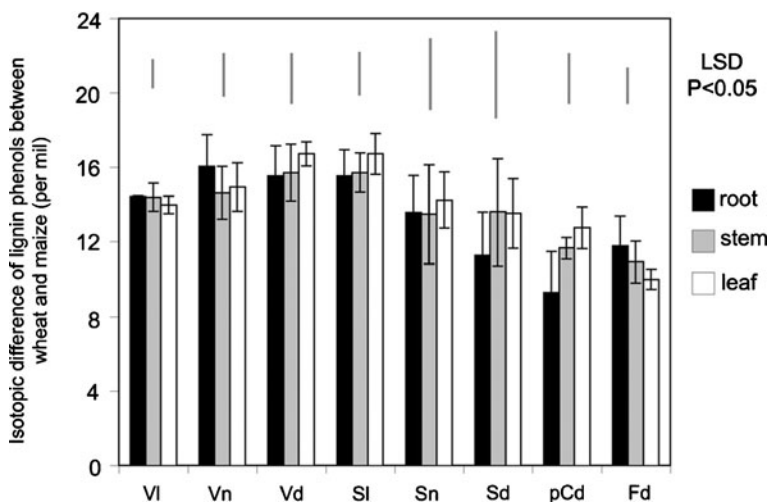
## Discussion

Lignin quantity and quality variation in the plants

The main differences in lignin content during the plant growth occurs during the grain filling phase. Decreases in dry matter in vegetative plant parts and C remobilization for grain filling has been observed in detail (Gallagher et al. 1976; Bidingner et al. 1977; Gebbing et al. 1998). Proteins and carbohydrates are mainly remobilized and marginally deposited (Gebbing et al. 1998), whereas lignin is only deposited in the cell walls and thus cannot be re-allocated to another part of the plant. This could explain its relative increase in the dry matter during the final step in maturity.

Regarding the plant organs, the root is the part containing the larger amount of lignin. This pattern is consistent with previous observations (Dignac et al. 2005; Heim and Schmidt 2007a; Abiven et al. 2005).

**Fig. 4** Isotopic differences between lignin monomers (VI Vanillin; Vn Acetovanillone; Vd Vanillic acid; SI Syringaldehyde; Sn Acetosyringone; Sd Syringic acid; pCd p-Coumaric acid; Fd Ferulic acid) of maize and wheat separated into plant parts (root, stem, leaf). Isotopic difference was calculated as  $\Delta = \delta^{13}\text{C}_{\text{maize}} - \delta^{13}\text{C}_{\text{wheat}}$ . The gray bars represent the LSD ( $p < 0.05$ ). Average values calculated from 3 replicates, black bars represent the standard errors





The qualitative importance of lignin in root structure has been already pointed out (Schreiber et al. 1999) but quantitative data are still sparse.

Roots are particularly enriched in C and S units compared to stems and leaves (Fig. 2, Table 2). A similar trend was observed in Dalzell et al. (2005) for senescent maize roots, stems and leaves. By contrast, differences between roots and stems were not so large for wheat and maize plants in Dignac et al. (2005). Using a thioacidolysis method, Schreiber et al. (1999) also observed the most prominent peaks for C and S units in the roots. The presence of C-type phenols in suberin (Kolattukudy 1980), typically more abundant in roots, could partially explain this result.

Thus the limited research to date has started to reveal a high degree of variability in lignin biosynthesis that may be related to conditions of growth (climate, water stress during plant growth) that affect the activity of the enzymes involved in lignin biosynthesis (Campbell and Sederoff 1996). The concentration and chemical quality of lignin monomers plant inputs variability should be assessed when calculating lignin monomers turnover.

Use of S/V and C/V ratios to trace the lignin in soil

S/V and C/V ratios have been found to be useful to characterize the origin of sedimentary organic matter (Hedges et al. 1986a, b). In our study, both the S/V and C/V ratios in the mature biomass decrease in the order: roots > stems > leaves, and maize > wheat at the harvest date (Fig. 2).

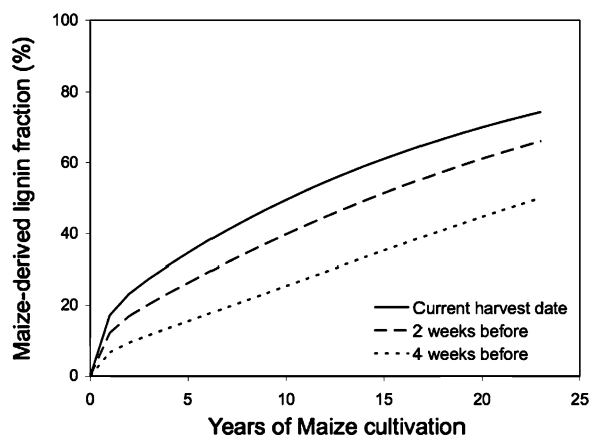
Table 3 summarizes S/V and C/V ratios of different grass parts from several previous studies, also at the harvest date. We report a large variability in the ratio values for a given plant part. For example, S/V and C/V ratios were calculated for maize in the four studies (Table 3—Lobe et al. 2002; Dignac et al. 2005; Dalzell et al. 2007; this study) ranging from 0.74 to 3.21 and from 1.92 to 6.20, respectively. This large range of observed values could be linked to our observations that C/V and S/V ratios varied during growth, and particularly during the filling of the grain.

We are proposing that variability observed between the studies is due to the growth stage when the plants were sampled, and, as a consequence, make the use of this C/V and S/V ratios tool potentially less powerful. Comparing different plants (angiosperm vs gymnosperm plants and grass, maize, wheat and sunflower), Thevenot et al. (2010) came to the same conclusion.

**Table 3** Literature review of C/V and S/V ratios in different grass parts

Plant	Plant part	C/V	S/V	Reference
Maize ( <i>Zea mays</i> )	Roots	4.19	3.21	Dalzell et al. 2007
	Stems	2.60	2.29	
	Leaves	1.42	1.38	
Mixed grasses ( <i>Cymbopogon</i> sp., <i>Themela</i> sp., <i>Setaria</i> sp., <i>Elionurus</i> sp.)	Roots	1.14	0.92	Lobe et al. 2002
	Shoots	1.89	0.99	
Maize ( <i>Zea mays</i> )	Roots	4.07	2.76	
	Shoots	3.46	1.99	
Wheat ( <i>Triticum aestivum</i> )	Roots	1.31	1.26	
	Shoots	1.00	1.28	
Wheat ( <i>Triticum aestivum</i> )	Roots	4.00	1.67	Dignac et al. 2005
	Stems	1.63	2.00	
	Leaves	1.27	1.36	
Maize ( <i>Zea mays</i> )	Roots	6.20	2.20	
	Stems	5.17	1.83	
	Leaves	2.50	1.60	
Wheat ( <i>Triticum aestivum</i> )	Roots	1.47	1.88	This study
	Stems	0.87	1.04	
	Leaves	0.54	0.59	
Maize ( <i>Zea mays</i> )	Roots	3.90	2.26	
	Stems	2.31	1.29	
	Leaves	1.92	0.74	

One would need to know the particular condition of the experimental set up and be able to measure the lignin biomarkers in the plants and in the soil to be able to use this ratio.



**Fig. 5** Modeled maize-derived lignin fraction as a function of time of maize cultivation under three inputs scenarios. Decomposition rates are identical for all three scenarios

However, in almost all the cases, both C/V and S/V ratios follow the order: roots > stems > leaves. If this pattern holds true, the S/V and C/V ratios could prove to be a tool to identify sources of soil organic matter from different plant organs.

The S/V ratio has been shown to decrease with decomposition in soil, indicating a selective loss of S-type lignin relative to V-type lignin (Ertel and Hedges 1984 in sediments; Kögel 1986 in forest soils) or to stay constant along the decomposition (Opsahl and Benner 1995). In our study, S/V ratios are larger in soil than in leaf and stem lignin, and lower than in the root lignin (Fig. 3). If we assume that S/V ratios decrease or stay stable along decomposition, our data can only be explained if root lignin is a major contributor to the soil lignin S/V ratio and so presumably to the soil lignin concentration.

Consequences of lignin content variability in plants for calculating soil lignin turnover

As described above, calculating the turnover of individual soil components using the natural abundance  $^{13}\text{C}$  labeling approach through  $\text{C}_3/\text{C}_4$  plants relies upon several assumptions: (1) lignin quantities in the two plants are identical during their growth, (2) lignin qualities are identical between the two plants, and (3) lignin distribution within the plant is homogenous, and (4)  $^{13}\text{C}$ -distribution in the lignin within each plant is homogeneous. Our results showed that, except for the last, all assumptions may be rejected, but what are the potential consequences of our results for calculating soil lignin turnover?

Using the model described in section 2.4. (Fig. 5), we found that after 23 years the fraction of maize-derived soil lignin may vary drastically between 74% (for the current harvest date), 66% (harvest 2 weeks earlier) and 50% (harvest 4 weeks earlier).

We may like to point out that these differences are large, and would translate into very different apparent turnover times of soil lignin using natural abundance labeling. Therefore it may be uncertain to conclude from the maize-derived lignin fraction on decomposition rates if the input variability is not known. On the other side, however, our calculations are a simplistic first attempt to estimate the effect of different amounts of lignin inputs. Inter-annual variations in biomass yield (and thus lignin input) average out over many years and actual variations might be smaller.

## Conclusions

We compared lignin quality and quantity in maize and wheat for individual plant parts during different growth stages.

- 1) Lignin concentrations increased particularly during the grain filling growth stage for both plants.
- 2) Lignin quality differed between plant species and plant parts systematically, i.e. for mature biomass S/V and C/V ratios both decreased in the order: roots > stems > leaves, and maize > wheat.
- 3) Roots had higher lignin concentrations than above ground parts, and contributed more to the soil lignin.
- 4) The distribution of the  $^{13}\text{C}$  was homogeneous within the plant parts of wheat and maize.
- 5) Systematic differences in harvesting time by a few weeks could change the proportions of maize-derived biomass drastically, and yield very different lignin turnover times, as a test with a simple model showed.

To summarize, the amount and chemical quality of lignin entering the soil may be very different between maize and wheat, depending on the time of harvest, and should be taken into account e.g. by analyzing harvested and remaining plant biomass, when calculating lignin turnover.

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**Authors contribution** The study was jointly conceived by A. Heim and M. Schmidt. Samuel Abiven carried out the analysis for the plant samples and A. Heim for the soil samples. S. Abiven and A. Heim analyzed the results and S. Abiven prepared the manuscript. All authors discussed the results and commented the manuscript.

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